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CELL-TO-CELL MICROFLUIDIC INTERACTION MODELS IN THE SCIENCE OF HEALTH

In the present review we provide the detailed discussion on the introduction of biological measures and on the nature of biological signals. The theory of absolute and relative biological measures has been briefly analyzed in case of mutual cell-cell and cell-tissue measurement. We focus on the biological signals and droplet microfluidic principles of cell-cell interaction modeling and biological measurements. Analysis of the theory of network organization with similar properties of structures and functions of biological particles is presented in the overview, and the theory of collective multilevel hierarchical organization that can form the basis of the description of the internal structures and relative biological measures is proposed. We further analyze possible applications of the discussed theoretical approaches to understand the measurement problems in modern biology and in the science of health.

Keywords: fundamental biological constants, biological measures, cell-to-cell measurement, droplet microfluidic models.

Introduction

Clinical diagnostics on human whole blood, plasma, serum, urine, saliva, sweat, and tears on a droplet microfluidic platform is one of the most promising applications for modern health and life sciences. Droplet-based mircrofluidic platform is a promising tool for investigation of human biological fluids widely applied in modern health and life sciences [28]. Repeatable high-speed transport of microdroplets of human physiological fluids is compatible with the electrowetting system [28]. Modern diagnostics is based on a droplet-based microfluidic technology that enables high-throughput single-cell processing. Single cells and reagents could be captured inside independent aqueous microdroplets (from 1 to 10 nL) dispersed in an immiscible carrier oil, allowing for digital manipulation of these reactors at a high rate [5]. Moreover, cell-to-cell microfluidic interaction models are the useful tool to investigate cell-cell and cell-tissue interactions on the model droplet systems and thus, to deepen understanding of the fundamentals behind the process and basics of the modern science of health.

Experimental investigation and modelling of the cell-to-cell interactions could be done using modern droplet-based microfluidics. However, there is a need to introduce standard measures in biology relative to which measurements are made and systems are compared to. In this paper we discuss the process of introduction the biological standard measures and fundamental constants and briefly overview the theoretical framework capable of dealing with the description of the entropy-induced forces, whose role in the cell organization is crucial.

Towards quantitative biological measurements

Cells in organisms interact and communicate in a highly complex and organized fashion. Cellular molecular organization is definitely not random, and its internal order could be represented as a hierarchically organized network. Most cellular functions are related to the groups of several functional molecules, not just to a single particular substance, i.e. cell could be modeled as a tightly interconnected modular network. Cell-to-cell interactions also are known to possess highly-ordered network character [9]. Moreover, cellular migration dynamics relevant to tissue repair, morphogenesis and tumor metastasis definitively has collective nature [29]: the traction force distribution near the leading shows non-Gaussian behavior and cannot be approximated as a leading-cell induced effect. Quantitative investigation of these interactions is just starting to appear, shifting from population-average measurements to single-cell analysis, which points out significant cell-to-cell variability [16]. Biological systems are complex and are unlikely to be made up of identical structural units:

diversity among individuals arises as a stabilizing mechanism. Interplay between nonlinearity, nonequilibrium and stochasticity determines dynamics at the levels of single cells and their inner compartments [19]. These developments require shift from descriptive to quantitative treatment, i.e. introduction of metrological basis to the biological sciences is needed in order to obtain accurate quantitative data, which could then be interpreted in an unambiguous way, as "there can be no exact science without exact data" [27]. In particular, the system biology is a newly emerging field, which is capable, at least in principle, to handle biological complexity and clarifies the connection between molecules and physiology [14].

Cell-to-cell interaction measures

Novel sensitive methods of studying the intercellular interactions [9] in vivo may lead to the introduction of the fundamental biological units of measurement similar to and in terms of the fundamental physical constants [18]. Precise quantitative biological measurements are urgently needed for toxicology, especially nanotoxicology [23,24] dealing with the investigation of nanomaterial's impact on cell function. It's important either to apply well-established toxicological methods and protocols to assess the toxicity of nanomaterials or to develop new cutting-edge procedures [23]. Some of these protocols have been developed in recent years [24]. These issues are directly related to health, and the underlying physical or biological mechanism of toxicity requires usage of appropriate techniques, among which the microfluidic diagnostic and modelling is a method of choice [12].

It's important to note that on the characteristic cell length scale (about 10-50 μm) water droplets behavior cannot be described by conventional approximations: though capillary water flow is laminar, cell-sized water flow is more likely to be a droplet-based. These cell-sized water droplets probably constitute the basic building blocks of liquid water at that length scale, as there is no way to imagine how capillary flow could be spontaneously disintegrated into droplets. The first step to better understand cell-to-cell interactions then should be related to the cell-sized water droplet dynamics and interaction analysis, which is pretty much possible to do using the microfluidic modeling techniques.

While there is now a plenty of ways to perform the measurements themselves, there is nevertheless lack of biological standards: we have no "standard biological cell", standard measure of the interaction strength between different cells, etc. There is a growing necessity to provide strict standardized fundamental definitions, interactions and constants as it is done in physics and chemistry in order to make biology much more quantitative science then it is now. The vast amount of available experimental data cannot be processed in an appropriate manner as there are no standards, relative to which measurements are made. Currently, in many cases, we can say that the concepts of control and reaction rates require more precise definitions in comparison with existing ones. Therefore, the increasing complexity of these systems require the standard biological measures to be global enough to capture that complexity and yet to have a particular degree of specificity in order to remain meaningful to the description of the systems of interest.

Cell-to-cell interactions should be distinguished according to their biological or purely physical nature: some effects may be related to the transport regulatory molecules through the intercellular connectors like plasmodesmata, tunneling nanotubes or septal pores [4]. However, some effects may be purely physical in nature and entropy-driven, as depletion interaction is one of the key driving forces leading to the colloidal structure organization inside cells. Protein folding and cell compartment organization in general is related to the so-called hydrophobic interaction being totally entropic in nature [25]. Osmotic potential resulting from differences in concentration profile of various ionic substances in cells is another example of physical effect having enormous impact on the functions and interaction of the particular cell. Indeed, if we are to understand the way how cells communicate and sense each other and such purely external physical quantities as the temperature and the pressure, there is a need to introduce strict biological standard measures.

Taking into account the current level of understanding the cell-to-cell interaction phenomena in terms of cooperative forces between cells and extracellular matrix (ECM) [17] and communication mechanism in plants, animals and fungi [4] it seems

that quantitative biological measures are likely to be developed soon. The huge (25,707 pages) basic encyclopedia of life sciences [8] allows here to discuss a few novel principal points in biological measures based on the introduction of the fundamental biological constants in this paper.

The absolute and relative biological measures

Definition of the fundamental biological constants is required in order to develop the biological metrology, which is based on standard biological measures. Related papers [13, 30] appear from time to time, but no systematic researches are still presented. However, these measures themselves are poorly defined and should be based on the fundamental biological constants. Not surprisingly, these fundamental biological constants also require strict definition. Few publications could be found by searching the appropriate keywords related to biological constants: we should mention [21], where the biological scaling relationships are employed using the term "biological constant", which, however, is neither constant nor universal at all and varies with size. Despite that, allometric (allometry = "different measure") scaling laws allow capturing the complexity of biological processes, when size dependence is considered [31]: scaling usually could be described by simple power-law model $Y = Y_0 M^b$, where Y is a biological quantity related to the mass of the organism M_b, by power-law behavior with exponent b and normalization constant Y_0. Another possible unifying phenomenon is the behavior of the exponent b. According to [31], the exponent b is usually a simple multiple of 1/4. Authors provide the following examples illustrating this unification: metabolic rate (b ~ 3/4), lifespan (b ~ 1/4), growth rate (b ~ −1/4), length of aortas and height of trees (b ~ 1/4), radii of aortas and tree trunks (b ~ 3/8), cerebral gray matter (b ~ 5/4), densities of mitochondria, chloroplasts and ribosomes (b ~ −1/4), and concentration of ribosomal RNA and metabolic enzymes (b ~ 1/4).

In fact, there are concerns about the validity of the model and the debate about the metabolic allometric scaling is still ongoing [32], and there is probably no single universal exponent. Nevertheless, the whole attempt to introduce some fundamental constant unifying vast variety of biological measurements is quite appealing and promising. Revelation of the (almost) universal scaling points out physical causes behind these relations and again raises the question of the distinction between purely biological and purely physical domains. Indeed, review of the physical aspects of fluid flow in capillary systems may clarify power-law behavior [22].

To sum it up, biological complexity contains both biological and physical contributions, so it's hardly possible to have any strict biological laws at all, and even if these laws would be strict, they probably would have little biology behind them. Biological complexity seems not to allow the existence of strict universal laws; however, the existence of strict biological measures and fundamental constants is not prohibited.

Entropic forces & hydrophobic effect

We begin our discussion with the analysis of entropy-driven interactions acting in cellular structures, considering the depletion interaction and the hydrophobic effect [15, 29]. Osmotic phenomena also have entropic nature. From the thermodynamical viewpoint every spontaneous process is associated with the net entropy growth, and solvent-solute interactions important to biomolecules immersed in cellular water are also driven by entropy. Conformation of polymers and shape of the colloidal particles in water environment is also determined by the entropic forces [1]. Hydrophobic effect determines vast amount of biologically relevant processes including protein folding and lipid bilayer dynamics in cell membranes.

The key concept behind the entropy-driven interactions lies pretty straightforward in the growth of total entropy of the system. It should be noted that in general no fundamental field could be associated with the entropic forces. To put in another way, if these forces have no potential it's impossible to model them using, for instance, conventional Hamiltonian formalism and the only way to make quantitative investigation is to use thermodynamical methodology. Molecular dynamics simulation usually indicates the correct in terms of entropy growth interaction of the solvent molecular ensembles and colloidal particles, but no mechanistic analytic treatment is available for these forces.

Hierarchical and network organization are intrinsic to cells, so it would be natural to use these terms in order to clarify their structural complexity. Entropic forces provide basis for the cell functioning and intercellular interactions. The underlying mechanism between cell-to-cell communication is now well-understood, but little is known on the mechanism behind intercellular interaction in a sense of cell measuring another cell and cell-tissue mutual measurement. In order to introduce strictly defined biological measures of these interactions there is a need to treat the entropic forces in a strict and deterministic manner. Recent developments suggest that entropic forces are causal in a sense of connection between adaptive behavior and entropy maximization [33]. Another major point is the usage of multiscale entropy method [6] to describe the complexity of biological signals. The point is that biological signals are neither stochastic nor absolutely organized, and cannot be described as one of these extremes.

While there is probably no way to treat the entropic forces using physical potentials, it's possible to model them as a consequence of semiempirical hierarchical potentials describing the interactions between adjacent levels of organization. If the physical potentials are clearly symmetric in terms of pairwise interactions, hierarchical potentials don't have to keep that property. The only way to apply analytic mechanistic formalism to describe these processes irreversible in a sense of thermodynamic spontaneity is to introduce asymmetric interaction potentials between objects of adjacent levels of organization. In this case there is an inequality between direct and reverse paths system can take, i.e. direct moving from the initial configuration *Xi* to the final configuration *Xf* is preferred over the reverse path due to the asymmetry of the driving force arising from the asymmetric potential acting between the levels of organization.

So, one and may be the only way to introduce analytic mechanistic description to the realm of entropic forces is switching to the hierarchical description with physical potentials acting on each organization level and semiempirical (and not necessarily symmetric) potentials between the adjacent levels of organization. We will discuss the basic theory behind the multilevel self-organization model in further sections. There is useful additional discussion of cell migration during development and cellular thermodynamics [8].

The entropy of living systems & biological signals

Taking into account the definition of the biological signal as the conditional entropic measure we consider here the general properties of cell signaling in living systems.

Information theory forms a suitable framework for biological applications. Entropy of living systems is defined as [20, 25] a measure of uncertainty of the distribution of states of the biological system :

$$H = -\sum_i p(x_i) \log p(x_i), \qquad (1)$$

where H is the Shannon entropy of the biological system, $p(x_i)$ is the probability to find system in the state i from x, summation is done over all number of states available to the system. Entropy of the living systems could be defined relative to distribution over any structural or functional indices and could be used to describe the biological organization systems.

Conditional entropy is defined as uncertainty of the distribution of states of the biological system relative to the known distribution :

$$H(x \mid y) = -\sum_{i,j} p(x_i, y_j) \log p(x_i \mid y_j), \qquad (2)$$

where $p(x_i \mid y_j)$ is the probability of finding system in a state i from x, while the reference system relative to which the uncertainty is measured is confined to states from y, $p(x_i \mid y_j)$ is the corresponding conditional probability.

Conditional entropy as a fundamental measure of the information content of biological system is extremely important when time evolution of the system of interest is considered. In that case reference probability distribution at time step t could be taken relative to the previous steps $t - 1$, $t - 2$, etc. If the number of available states or the volume of the phase space available to the system doesn't change with time, the conditional entropy of the distribution related to the reference distribution by the simple relationship (3):

$$H(x \mid y) = -\sum_i p(x_i) \log p(x_i, y_j), \qquad (2)$$

Relative entropy is positive, equals zero only if $p(x_i) = p_i$ and is also a convex function of x_i [20].

In general, every change in living organism is associated with the corresponding entropy change. Definition of the conditional entropy according to eq. and eq. allows us to clarify the underlying physical basis behind the differences cells sense when they measure other cells. In order to capture the dynamics of the system transition rather than static probabilities should be introduced [25]. The conditional probability to find x in state $x(i+1)$ at time $t(i+1)$ is $p(x(i+1)|x(i),x(i-1),...,x(i-k+1))$ if the system could be approximated by a stationary Markov process of order k. Using the shorthand notation $i_n^{(k)} = (x(n), x(n-1),...,x(n-k+1))$ we define the cell-cell measurement act as a process of information transfer from cell J to cell I quantitatively described by the corresponding transfer entropy :

$$T_{J\to I} = -\sum_n p(i_{n+1}, i_n^{(k)}, j_n^{(l)}) \times \quad (4)$$

$$\times \log \{p(i_{n+1}|i_n^{(k)}, j_n^{(l)}) / p(i_{n+1}|i_n^{(k)})\}.$$

Application of entropic measure can be extended to specific biological measures of cell biology. We will discuss some examples below.

Hierarchical self-organization measure

Let's take a look at the problem of introducing the hierarchical measures of multilevel collective self-organization in an analytic framework based on the concept of auxiliary biological particles (Fig.1). These auxiliary particles (or units of organization) are added to the system at the particular time and to the right place, and their properties are predetermined by the mathematical birth or destruction rules and by the inheritance rules depending both on the properties of the parent level objects and of all phases of the system (Fig.1,2) [7].

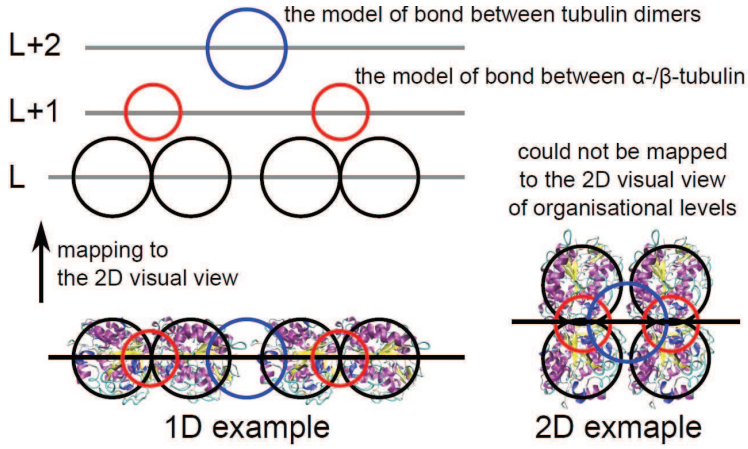

Fig. 1. The principle of establishing bonds as auxilary particles on the example of the longitudinal and lateral bonds of the tubulin protein dimers

This approach could be formulated in Hamilton formalism if we introduce the following Hamiltonian (5):

$$\begin{cases} H = \sum_{i,j} \mathrm{T}_j^i \left(\frac{(p_j^i)^2}{2m_j^i} + \sum_{k\neq i} \mathrm{T}_j^k \frac{1}{2} U_j^L(q_j^i, q_j^k) + \right. \\ + \sum_{k\notin D_j^i} \mathrm{T}_{j+1}^k \frac{1}{2} U_j^{L+1}(q_j^i, q_{j+1}^k) + \sum_{k\notin P_j^i} \mathrm{T}_{j-1}^k \frac{1}{2} U_j^{L-1}(q_j^i, q_{j-1}^k) + \\ \left. + \sum_{k\in P_j^i} \mathrm{T}_{j-1}^k \frac{1}{2} U_j^P(q_j^i, q_{j-1}^k) + \sum_{k\in D_j^i} \mathrm{T}_{j+1}^k \frac{1}{2} U_j^D(q_j^i, q_{j+1}^k) \right), \end{cases}$$

where j – organization level number; i – the number of bioparticle; q_i^j – coordinates; p_j^i – impulses; m_j^i – the mass of i particle j level; U_j^L – interaction potential between particles of j level; U_j^{L+1} – particles of level j and $j+1$; U_j^{L-1} – particles of level j and $j-1$; U_j^P – particles of level j with "parent" particles of $j-1$; U_j^D – particles of level j with "child" particles of level $j+1$; P_j^i – denotes group of "parents" for i particle of j level; D_j^i – denotes group of "child" for i particle of j level; T_j^i – is a function (superposition of theta-functions) which is define the existence of unit of organization.

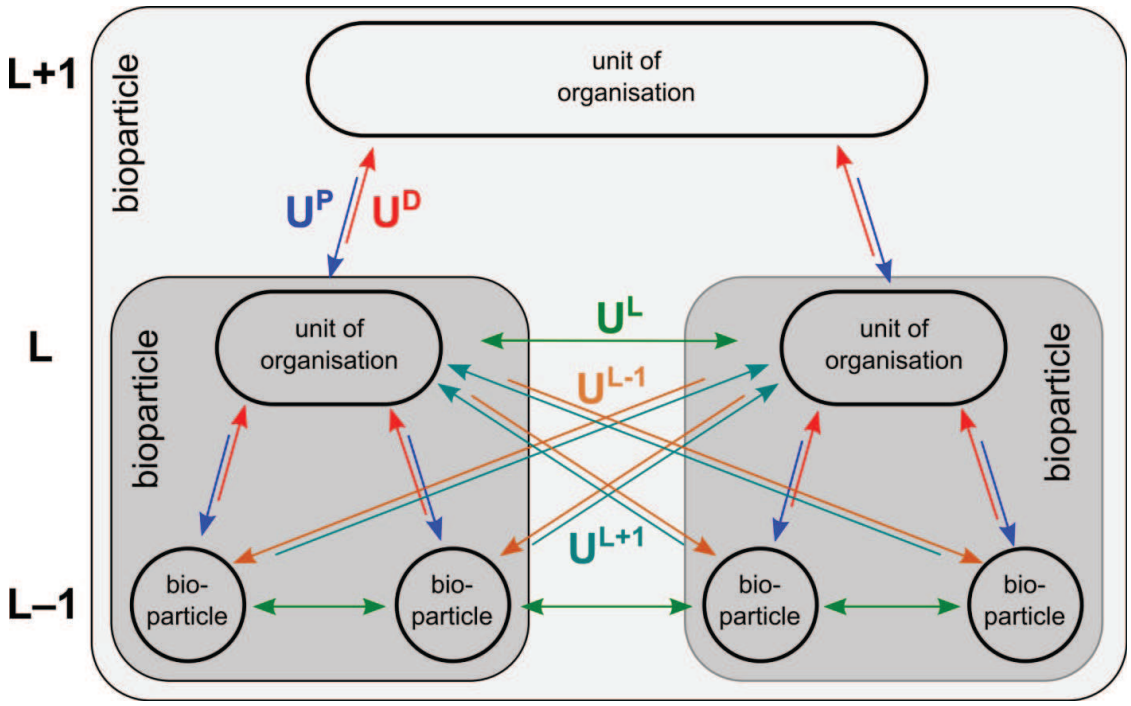

Fig. 2. Three-level system and five potentials for the description multilevel collective self-organization **of biological particles**

Examples of numerical solutions of equation (5) for a system of multiple objects and the set of potentials, triggers and successors are shown in Fig. 3. These solutions were obtained using the specially developed application. This example illustrates the possibility of construction the biological measures of algorithmic relationships in such processes occurring in the cell, as the self-assembly of cytoskeleton filaments and the coil-globule transformation.

Investigation of collective motions and energy transfer between levels in a multilevel model (Fig. 3) is useful for the introduction of hierarchical steps in cell biology. The trigger activates the corresponding intra and inter-level potentials in the Hamiltonian, changing the total energy of the system, as well as the energy landscape. Further dynamics includes a new object with properties that define the successor function. Application for exploration of suggested model, described by eq. 5, is open for download [7] and can be freely used for the research of different hierarchical self-organization measures with contextual biological potentials.

Droplet microfludic measures

Nowadays droplet-based microfluidics is usually called ‘’digital microfluidics’’. The area faces rapid growth in recent years, and these technologies are promising tools to study the fundamental laws of liquid dynamics below the capillary regime. Bulk liquid behavior in laminar regime down to characteristic capillary length scale has well-developed theoretical base, while fluid transport in cell-sized channels and systems is less familiar. Strict theoretical treatment is needed to understand fluid flow at the cell-sized length scale, and droplet-based microfluidics offers huge variety of opportunities to develop the model. The theory of fluid flow in cell-sized structures should clarify vast amount of experimental data available on e.g. water transport in plants and intercellular interactions.

Clinical diagnostics of human physiological fluids have started from a droplet-based microfluidic platform [5, 28]. Microfluidic principles of biological measurements have been intensively developed and allows introduction of biological measures

useful for assessment of cell-cell measuring acts. Recent results and protocols in [3] show growing tendency towards shift to single cell investigation and two-dimensional manipulation of droplets with controllable interaction [3].

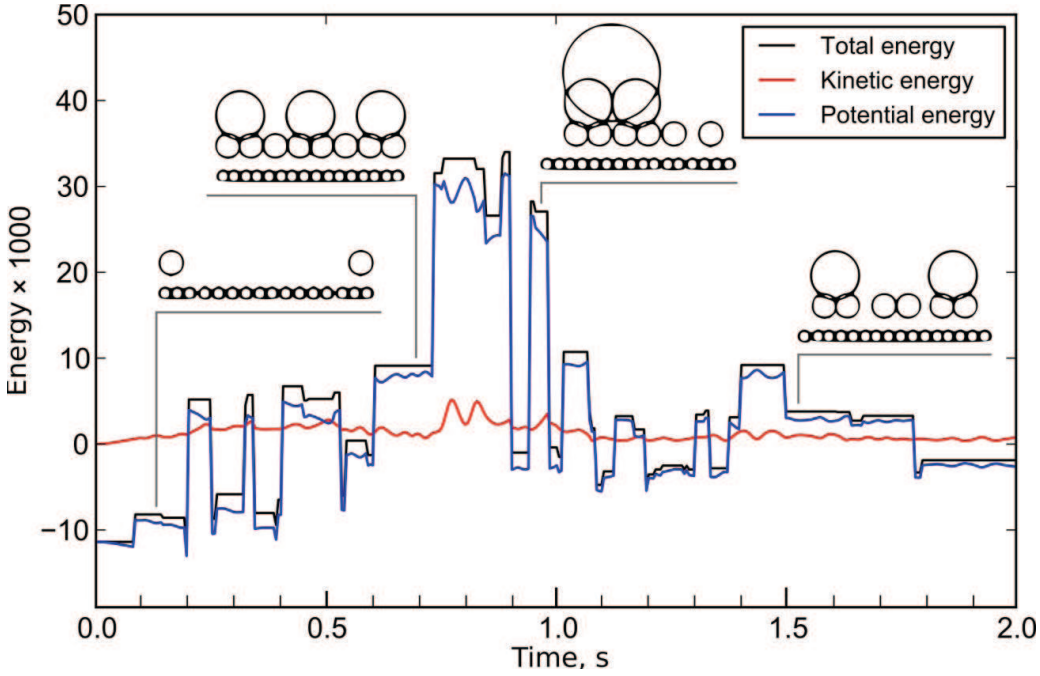

Fig.3. Example of hierarchical self-organization measure is based on eq. (5)

The simplest approximation to cell is a water droplet with e.g. ionic contents covered with surfactant. Study of the transport and interaction between these droplets may reveal the underlying physical mechanisms in cell-cell communications. Among new advanced methods available now for droplet manipulation we should mention controlled droplet generation, fusion, sorting, splitting and storage [26].

Introduction of biological measures for cytoskeleton is a difficult subject for this short paper, so we make very short discussion on cytoskeleton in connection with the droplet microfluidics and emphasize artificially induced actin behavior at cell-sized water droplet (CWD) [10]. In [10] authors have conducted microscopic observations on the structural changes in actin filaments in a cell-sized (several tens of micrometers in diameter) water droplet coated with a phospholipid membrane as a simple model of a living cell membrane. Their results show that depending on the magnesium ion concentration in the water phase actin filaments are either uniformly distributed or adsorbed onto the inner membrane surface. These results suggest, as authors have pointed out, that a microscopic water droplet coated with phospholipid can serve as an easy-to-handle model of cell membranes [10]. Of course, in order to obtain quantitative biological measures for different cytoskeleton phases, thorough analysis of [7] should be performed, when fine details of phases and states of the cells are taken into account.

Conclusion

Consideration of the examples provided in this paper from cellular biology and epigenetics allows us to conclude that the ground for introduction of new fundamental biological constants would appear soon. We define the cell-to-cell communication act as an information transfer process characterized by the corresponding transfer entropy. Realization of quantitative measurements of suitable quality should be done using modern cell microfluidics techniques as they are promising in providing

insight into intercellular communications through simple yet precise and illustrative models.

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